DISSOCIATION IN AN ENCAPSULATED STAPHYLOCOCCUS

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The organism to be described was isolated from an autopsy at the Johns Hopkins Hospital in April, 1929. The case was that of a young colored man with acute ulcerative gonococcal endocarditis. A typical *Staphylococcus aureus* was isolated from the heart blood and an encapsulated strain from the pericardial and the peritoneal fluids, despite the fact that there was no gross or microscopic peritonitis or pericarditis. Bacterial stains of the pericardium showed no organisms, and sections of the heart valve showed only Gram-negative diplococci.

The organisms are fairly large Gram-positive cocci. In broth (fig. 1) culture they occur most regularly singly, in pairs and occasionally in very small clusters, large groups being rarely seen except from solid media (fig. 2). They are surrounded by a well defined and extensive capsule which may include two organisms. The cocci and surrounding capsule measure 3 microns. Owing to the fact that the capsules are very soft and viscid and spread over the surface of the slide, retaining no definite outline in stained preparations, they are best observed when suspended in India ink (fig. 3).

The organisms grow well on all laboratory media. On blood agar, as well as plain infusion agar, the colonies are characteristic of those of an encapsulated organism. They are large, confluent and well elevated above the agar, with a smooth glistening surface and entire edge (fig. 4a). The colonies are more translucent than those of a typical staphylococcus and are very mucoid and tenacious, adhering to the needle. On blood agar, the surface colonies

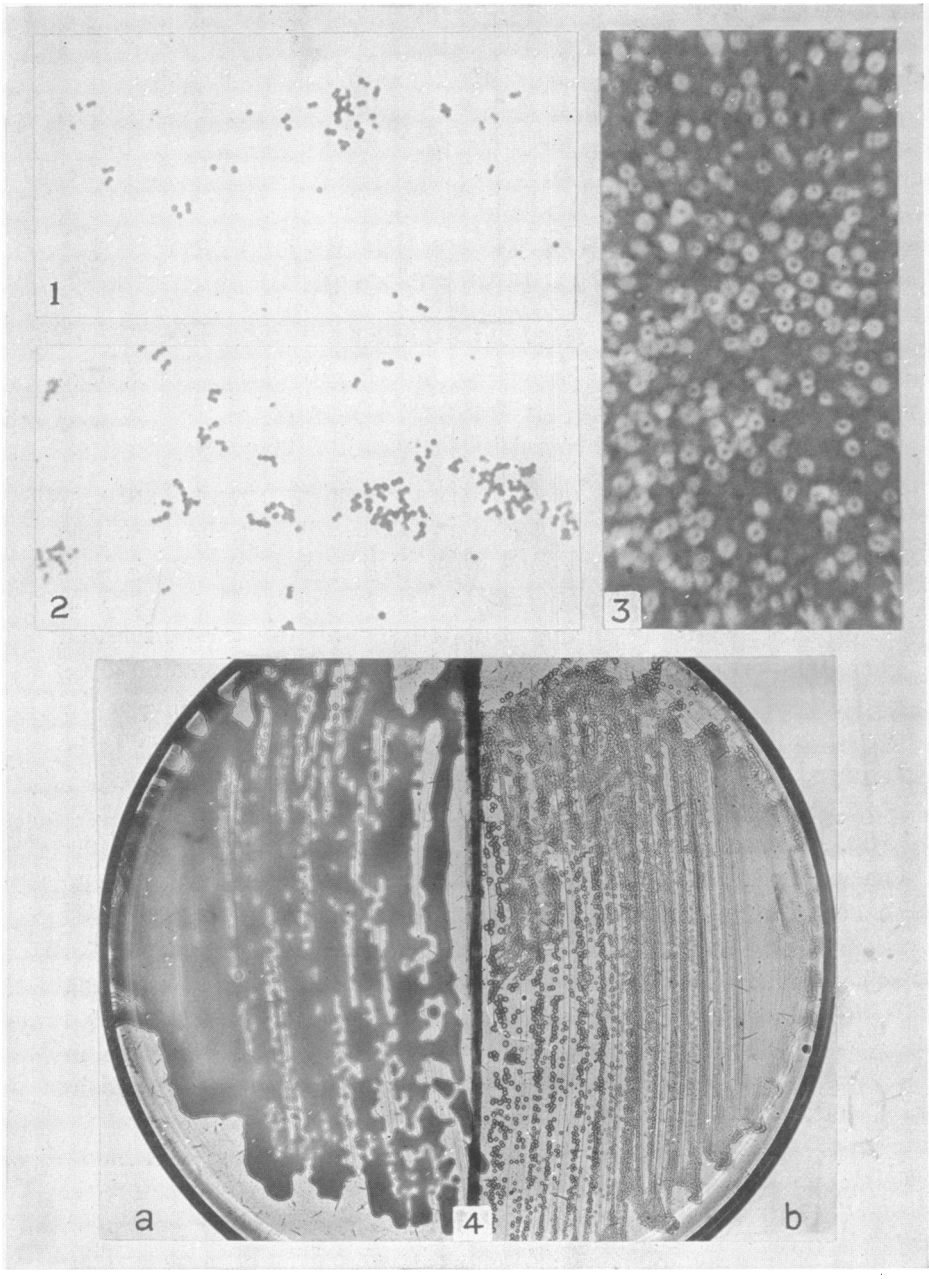

FIG. 1. GRAM STAIN OF BROTH CULTURE. ×1000

FIG. 2. GRAM STAIN OF AGAR CULTURE. ×1000

FIG. 3. MOIST INDIA INK PREPARATION OF S STRAIN SHOWING CAPSULES

FIG. 4. INFUSION AGAR STREAK PLATE AFTER INCUBATION FOR TWENTY-FOUR HOURS

a, S strain; *b*, R strain

show Beta hemolysis, while the deep colonies, which are large, biconvex and opaque, are non-hemolytic. The growth on an infusion agar slant, on Loeffler's coagulated serum slant and on potato is heavy, spreading, smooth and glistening and, on the latter two, shows a brilliant orange color.

There is a heavy growth in infusion broth after twenty-four hours incubation, with the production of a wide yellowish-white ring at the surface of the media which in old cultures becomes bright orange in color. An abundant and very viscid sediment is formed and the culture as a whole is very thick and mucoid.

On gelatin the organism produces saccate liquefaction. In milk plus brom cresol purple the reaction is alkaline with the production of a soft curd and slowly progressive peptonization. Indol is not formed but nitrates are reduced to nitrites. Glucose, maltose, sucrose, levulose, galactose and inulin are fermented while lactose, mannitol, xylose, starch, raffinose, salicin, dulcitol, dextrin and rhamnose are not.

One month after the isolation of the encapsulated strain, small, white, opaque secondary colonies appeared on the surface of the infusion agar slants and Loeffler's slants kept at ice-box and incubator temperature. When these cultures are plated out, two types of colonies are observed. The first type is identical with those of the encapsulated strain. This strain has been called the S strain (fig. 4a). The second type is a small, round, opaque, discrete, morphologically typical staphylococcus colony (fig. 4b). No capsules can be demonstrated from these colonies or on any sub-cultures from this strain, which has been called the R strain. Dissociation into R and S forms takes place in all cultures, broth, agar slant, Loeffler's slants and milk which are from fifteen to twenty days old, but more readily in broth culture than on solid media, and at incubator temperature rather than at ice-box temperature.

Morphologically and culturally the R type organisms are identical with the S strain, with the exception of those properties which depend upon the presence of a capsule. They also occur most regularly singly, in pairs and in very small clusters and no large groups are seen except from solid media. They differ from

the S form in their opacity, and soft cheese-like consistency. On the other hand, they differ from a stock strain of *Staphylococcus aureus* in that occasionally, when injected into guinea pigs, they revert to the S form.

The encapsulated S strain is highly virulent for guinea pigs, 0.025 cc. of a twenty-four-hour broth culture injected intraperitoneally killing within twenty-four hours; while 5 cc. of the R strain produces no effect. The stock S strain used in determining the virulence is one which has been kept at ice-box temperature for a year and subcultured monthly, at each subculture, plates having been made to insure the purity of the strain and to eliminate the presence of any of the unencapsulated organisms which might have been present due to dissociation. This strain has never been passed through guinea pigs. The stock R strain used is one which has also been kept at ice-box temperature since dissociation first occurred. It has been subcultured monthly, plates having been made at each subculture and no encapsulated organisms have ever appeared. This strain also has never been passed through animals.

Large quantities of this R strain, 8 to 10 cc., occasionally kill guinea pigs. If death occurs within twenty-four hours, cultures of the heart blood and the peritoneal fluid contain only unencapsulated staphylococci in pure culture. If death occurs after forty-eight hours or if pigs which have not died in twenty-four hours are killed, cultures of the peritoneal fluid and the heart blood show both R and S forms. Control plates have been made of the R culture injected and no encapsulated organisms found. The virulence of the encapsulated strain as obtained from R by animal passage is equal to that of the original encapsulated strain.

SUMMARY

A strain of encapsulated *Staphylococcus aureus* has been described. This strain, which is highly virulent for guinea pigs, dissociates into an unencapsulated relatively avirulent strain. Reversion of the unencapsulated to encapsulated form may be brought about by passage through a guinea pig. Further work is being done in this laboratory on the antigenic and surface properties of these two strains.